FEBS Letters 581 (2007) 3909–3914

Novel clusters of highly expressed genes accompany genomic amplification in breast cancers

Emi Ito[a], Reiko Honma[a], Yuka Yanagisawa[a], Jun-ichi Imai[a,b], Sakura Azuma[c], Tetsunari Oyama[d], Susumu Ohwada[e], Tetsu Akiyama[f], Nobuo Nomura[b], Jun-ichiro Inoue[c], Shinya Watanabe[a,b], Kentaro Semba[c,g,h,*]

[a] *Department of Clinical Informatics, Tokyo Medical and Dental University School of Medicine, Tokyo, Japan*
[b] *Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan*
[c] *Division of Cellular and Molecular Biology, Department of Cancer Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*
[d] *Department of Pathology, Dokkyo Medical University School of Medicine, Mibu, Japan*
[e] *Department of Surgery, Gunma University, Graduate School of Medicine, Gunma, Japan*
[f] *Laboratory of Molecular and Genetic Information, Institute for Molecular and Cellular Biosciences, The University of Tokyo, Japan*
[g] *Institute for Biomedical Engineering, Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Tokyo, Japan*
[h] *Department of Life Science and Medical Bio-Science, Waseda University, 3-4-1, Okubo, Shinjuku-ku, Tokyo 169-8555, Japan*

Received 19 June 2007; revised 30 June 2007; accepted 6 July 2007

Available online 23 July 2007

Edited by Takashi Gojobori

Abstract **Breast cancer is the most common cancer in women worldwide. To identify novel amplicons involved in the mammary carcinogenesis, we constructed gene expression maps of chromosomes in 35 human breast cancer cell lines and extracted six candidate amplicons containing highly expressed gene clusters on chromosomes 8, 17, and X. We also confirmed the presence of the identified amplicons in clinical specimens by Southern blot analysis. Highly expressed genes identified in the amplicons will contribute to the characterization of breast cancer phenotypes, thereby providing novel targets for anticancer therapies.**

Keywords: Breast cancer; Gene amplification; Microarray

1. Introduction

Cytogenetic and molecular studies on breast cancer have frequently identified imbalanced chromosomal regions. Several amplicons and candidate genes have been identified therein, including 8q24 (*MYC*); 8p11-12 (*SPFH2*, *BRF2*, and *RAB11-FIP1*); 11q13 (*CCND1*, *CTTN* [*EMS1*], *C11orf30* [*EMSY*], *PAK1*, and *THRSP*); 12q13 (*MDM2*); 17q12-21 (*ERBB2*); 17q23 (*RPS6KB1*, *TRIM37* [*MUL*], *APPBP2*, *THRAP1* [*TRAP240*], *RAD51C*, and *BCAS3*); and 20q11-q13 (*NCOA3* [*AIB1*], *AURKA* [*BTAK*], *BCAS1* [*NABC1*], *ZNF217*, and *BCAS4*) [1–3]. One of the best-characterized genes that are amplified in breast cancer is the *ERBB2* (*HER2/NEU*) gene, a member of the epidermal growth factor receptor family. Overexpression of *ERBB2* due to gene amplification is observed in almost 30% of breast cancers, and *ERBB2* has been used as a diagnostic marker and an indicator of poor prognosis in breast cancer [4–7]. *ERBB2* is also a clinically relevant target for the treatment of breast cancer. Therapy with humanized monoclonal antibodies against *ERBB2* (Herceptin™, Genentech, Inc.), either alone or in combination with conventional chemotherapeutic agents, is effective against a subset of *ERBB2*-overexpressing breast cancers [8,9]. Thus, it is biologically and clinically important to identify genomic alterations in tumors and study the functions of the genes involved. Recent microarray-based comparative genomic hybridization (CGH) combined with expression analysis has accelerated the identification of novel amplicons and candidate genes, some of which have been found to be associated with tumorigenesis [3,10–12]. In this study, we describe novel amplicon candidates that were explored using gene expression profiles obtained from breast cancer cell lines and whose presence was confirmed in clinical specimens.

2. Materials and methods

2.1. Cell lines

Human breast cancer cell lines were obtained from the Cell Resource Center for Biomedical Research, Tohoku University (CRL1500, MCF7, SK-BR-3, and YMB-1-E), and purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA; http://www.atcc.org/) (BT-20, BT-474, BT-483, BT-549, CAMA-1, DU4475, HCC1143, HCC1395, HCC1419, HCC1500, HCC1937, HCC1954, HCC202, HCC2157, HCC2218, HCC38, HCC70, Hs 578T, MDA-MB-134VI, MDA-MB-157, MDA-MB-175VII, MDA-MB-231, MDA-MB-361, MDA-MB-415, MDA-MB-436, MDA-MB-453, MDA-MB-468, T-47D, UACC-812, UACC-893, and ZR-75-30). All cell lines were cultured according to the recommendations of the supplier.

2.2. Microarray analysis

Synthetic polynucleotides (80-mers) representing 30913 species of human transcripts (MicroDiagnostic, Tokyo, Japan) were arrayed by using a custom arrayer. Poly(A)$^+$ RNA was extracted from cells harvested at approximately 70% confluency after incubation in fresh media for 16 h. Two micrograms of poly(A)$^+$ RNA was labeled with Cyanine 5-dUTP or Cyanine 3-dUTP (PerkinElmer, MA, USA). Human common reference RNA was prepared by mixing equal amounts of poly(A)$^+$ RNA extracted from 22 human cancer cell lines to reduce

*Corresponding author. Address: Department of Life Science and Medical Bio-Science, Waseda University, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Fax: +81 3 5449 5278.
E-mail address: ksemba@waseda.jp (K. Semba).

Abbreviations: CGH, comparative genomic hybridization

0014-5793/$32.00
doi:10.1016/j.febslet.2007.07.016

cell type-specific bias of expression [13]. Hybridization was performed with a labeling and hybridization kit (MicroDiagnostic). Signals were measured with a GenePix 4000A scanner (Axon Instruments Inc., CA, USA) and then processed into primary expression ratios (ratio of the Cyanine-5 intensity of each cell line to the Cyanine-3 intensity of the human common reference RNA). Each ratio was normalized by multiplication with the normalization factors using the Gene Pix Pro 3.0 software (Axon Instruments Inc.). The primary expression ratios were converted into $\log_2$ values (designated log ratios). The mean average log ratio of 35 cancer cell lines was calculated for each transcript; this was then subtracted from the log ratio for each transcript (designated subtracted log ratio). The subtracted log ratios were used as the final data for subsequent amplicon analysis (Supplementary Table 1). We assigned an expression ratio of 1 (log ratio of 0) for spots that exhibited fluorescence intensities under the detection limits, and we did not include these in the signal calculation of the mean averages and subtracted log ratios. The data were processed using Microsoft Excel software (Microsoft). In accordance with the Minimum Information about a Microarray experiment (MIAME) guideline, all the data were deposited at the DNA Data Bank of Japan (DDBJ) via the Center for Information Biology gene EXpression (CIBEX) database (http://cibex.nig.ac.jp/index.jsp) under the accession number CBX20.

2.3. Construction of expression maps

Gene names, gene abbreviations, and accession numbers were obtained from the Human Genome Organization (HUGO) Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/index.html) [14]; information for gene localization on chromosomes was acquired from GeneLoc (http://genecards.weizmann.ac.il/geneloc) [15]. From a total of 30913 species of transcripts on the microarrays, we generated high-resolution expression maps based on the information obtained for 17373 transcripts for which localization information was available. For each chromosome of each cell line, the subtracted log ratios were plotted on the vertical axis versus the initiation sites of the transcripts on the horizontal axis.

2.4. Breast cancer DNA and Southern blot analysis

DNA samples extracted from the cell lines and tumor specimens were subjected to standard Southern blot analysis. Human breast cancer specimens were obtained from the Dokkyo Medical University Hospital. The ethics committee of the Dokkyo Medical University approved this study, and informed consent was obtained from all patients. Complementary DNA (cDNA) fragments for the probes were amplified by PCR and they were then used directly or cloned into pGEM-Teasy (Promega) for labeling. Information regarding each probe is available from the corresponding author. Human DRG-1 cDNA at 22q12.2 was used as a control. The fragment size detected by each probe was confirmed by genomic information available at http://www.ensembl.org/Homo_sapiens/index.html.

3. Results and discussion

To explore gene clusters whose expression levels abnormally increased in cancer based on analyses of gene expression profiles, we constructed an analytical system to identify such gene clusters and validated this system on the basis of previously identified gene clusters. Since elevated expression of *ERBB2* and its neighboring genes in the chromosomal region of 17q12-21 has been extensively analyzed in breast cancer, we first focused on gene expression in chromosome 17 among the 35 breast cancer cell lines. We obtained the gene expression profiles of these cell lines as ratios against the human common reference RNA [13] (designated log ratios), extracted the expression data for genes localized on chromosome 17, and assembled the data according to the position on the chromosome to clarify the relationship between expression ratios and chromosomal positions. Thereafter, to emphasize the presence of gene clusters with elevated expression levels, we processed each log ratio of gene expression into a log value relative to the mean average log ratio of each gene among 35 profiles (designated subtracted log ratios). Further, we plotted the subtracted log ratios on the vertical axis and the initiation sites of the transcripts on the horizontal axis (see Section 2 for calculation details). Fig. 1 shows that the subtracted log ratios of most transcripts are plotted around the origin of the vertical axis (i.e., the mean average log ratio). In contrast, several dots comprise spike-like clusters with higher expression levels than the mean average for each gene among the 35 cell lines. Among these spike-like clusters, we confirmed the presence of three amplicons previously identified in breast cancer (Fig. 1, blue arrows): at 17q12-21, contain-

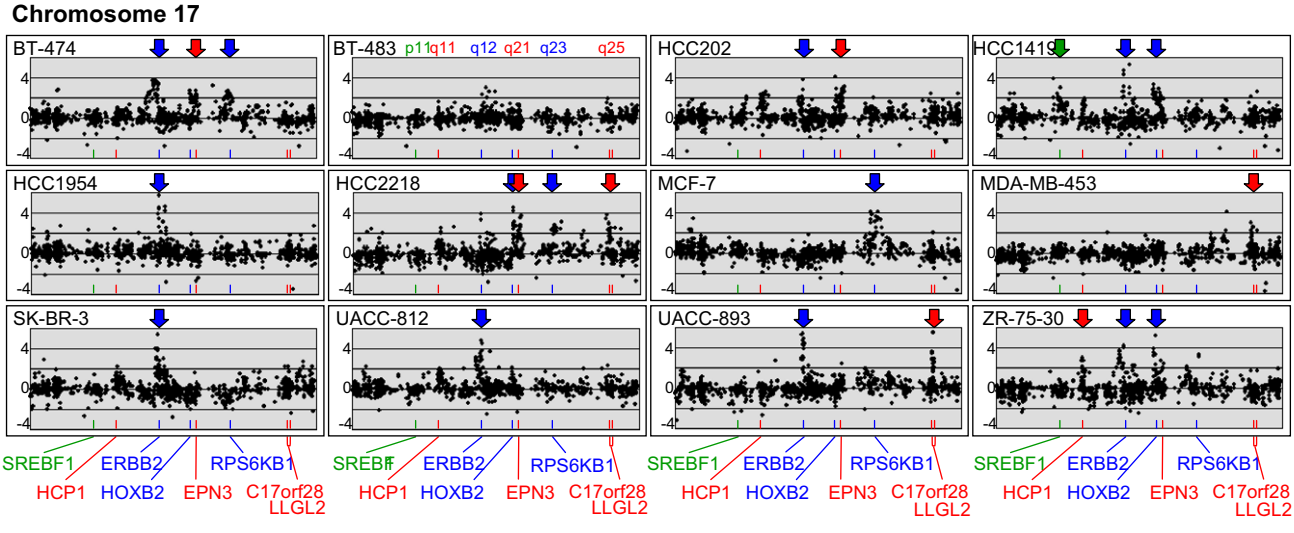

Fig. 1. Mapping of gene expression data on chromosome 17. Eleven "amplicon-positive" cell lines with novel candidates or previously identified amplicons and one "amplicon-negative" cell line. The maximum values on the horizontal axis are proportionally equivalent to the sizes of the chromosomes, 79 Mb. The arrows indicate the amplicons: red, novel amplicon candidates; green, amplicons not identified in breast cancer so far but reported in other cancers previously; and blue, previously reported amplicons. The panel for BT483 cells includes the number of bands for each chromosome (see Supplementary Fig. 1 in terms of 23 other "amplicon-negative" cell lines).

ing *ERBB2*, in BT-474, HCC202, HCC1419, HCC1954, SK-BR-3, UACC-812, UACC-893, and ZR-75-30 cells [16]; at 17q21-22, containing *HOXB2*, in HCC1419, HCC2218, and ZR-75-30 cells [17]; and at 17q23, containing ribosomal protein S6 kinase (*RPS6KB1*), in BT-474, HCC2218, and MCF-7 cells [17].

Since we successfully observed previously identified amplicons as spike-like clusters in the plots described above, we next screened novel amplicon candidates under more precisely defined conditions with several parameters. Using conditions that were satisfied by the previously identified amplicons, we defined the new screening condition as follows: (1) assigning a 0.5-Mbp region for each transcript wherein its initiation site is centrally localized; (2) extracting candidates in this assigned region, which comprise clusters of transcripts with mean average subtracted log ratios greater than 1.0; and (3) selecting the candidates which contain at least 4 transcripts with subtracted log ratios greater than 1.8. When multiple transcripts harbored identical initiation sites, we selected a single representative transcript. Moreover, when the candidates comprised members of identical gene families only, we abandoned these candidates since an identical mechanism may modulate highly elevated expression of such transcripts, irrespective of gene amplification. Our microarrays provided information of 11.9 transcripts on average in each 0.5-Mbp region of chromosome 17; 2.2% transcripts exhibited subtracted log ratios greater than 1.8 throughout chromosome 17. Eventually, on screening chromosome 17 under these conditions, we identified three novel amplicon candidates at 17q11 in ZR-75-30 cells; at 17q21 in BT-474, HCC202, and HCC2218 cells; at 17q25 in HCC2218, MDA-MB-453, and UACC893 cells (Fig. 1, red arrows). Moreover, an amplicon candidate detected at 17p11 in HCC1419 cells, containing *SREBF1*, has not been identified in breast cancer so far but reported in leiomyosarcomas previously (Fig. 1, green arrow) [18]. Fig. 2 shows detailed descriptions of subtracted log ratio values for these amplicon candidates.

Since we successfully detected novel and previously identified amplicons in chromosome 17, we extended the application of the existing screening approach to chromosomes 8 and X, and the results obtained are demonstrated in Fig. 3. In case of chromosome 8 and X, 6.0 and 6.2 transcripts on average are contained in each 0.5-Mbp region; 2.5% and 2.0% of transcripts exhibited subtracted log ratios greater than 1.8, respectively. By integrating gene expression information of each 0.5-Mbp region, we identified three novel amplicon candidates at 8q13 in HCC2157 cells, at 8q21 in HCC1419 cells, and at

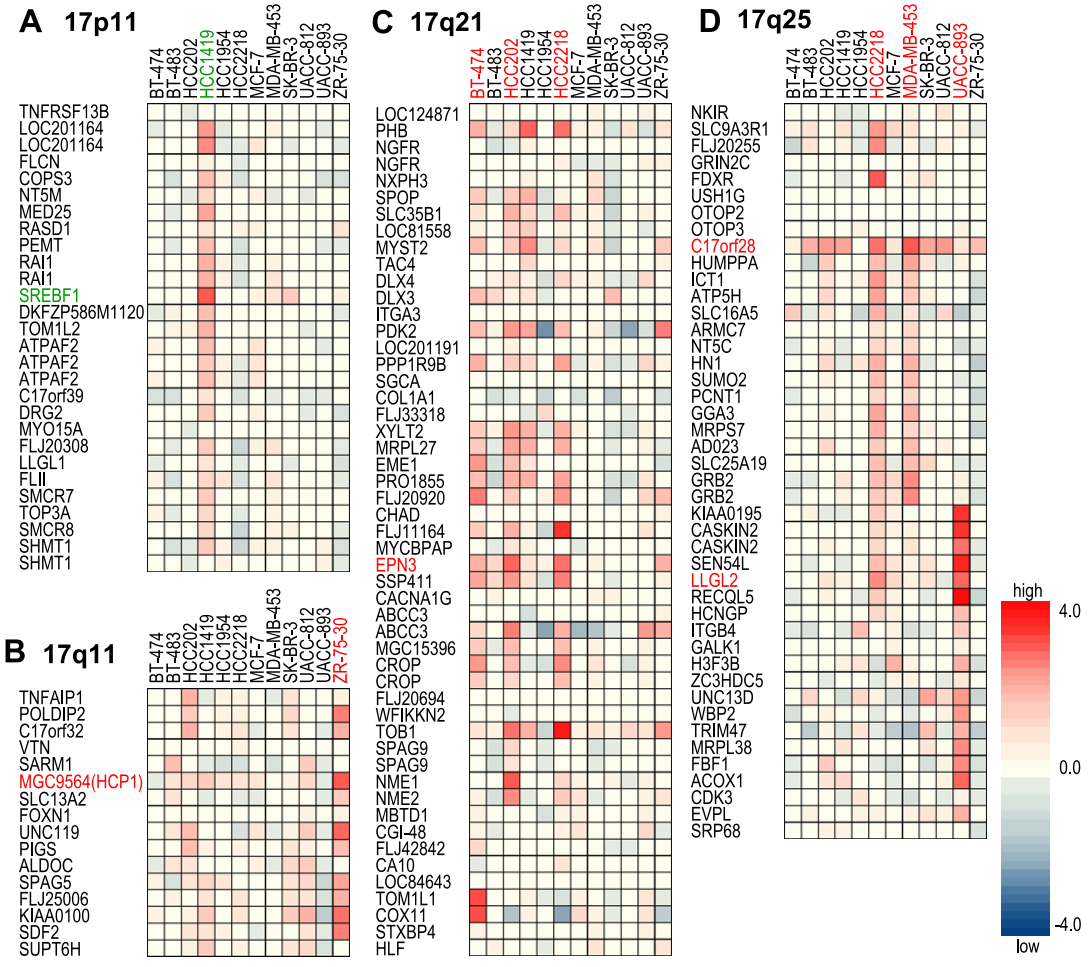

Fig. 2. Relative expression levels of genes localized in amplicon candidates detected in chromosome 17. Expression values (subtracted log ratios) of the genes localized at 17p11 (A), 17q11 (B), 17q21 (C), and 17q25 (D) are represented in color. Cell lines depicted in red and green contain spike-like clusters shown in Fig. 1 as red or green arrows. Genes are aligned in order of chromosome location. In the color bar shown at the bottom right, red and blue indicate high and low expression levels relative to mean average log ratio for each probe, respectively. Gradations of the two colors indicate changes in relative gene expression.

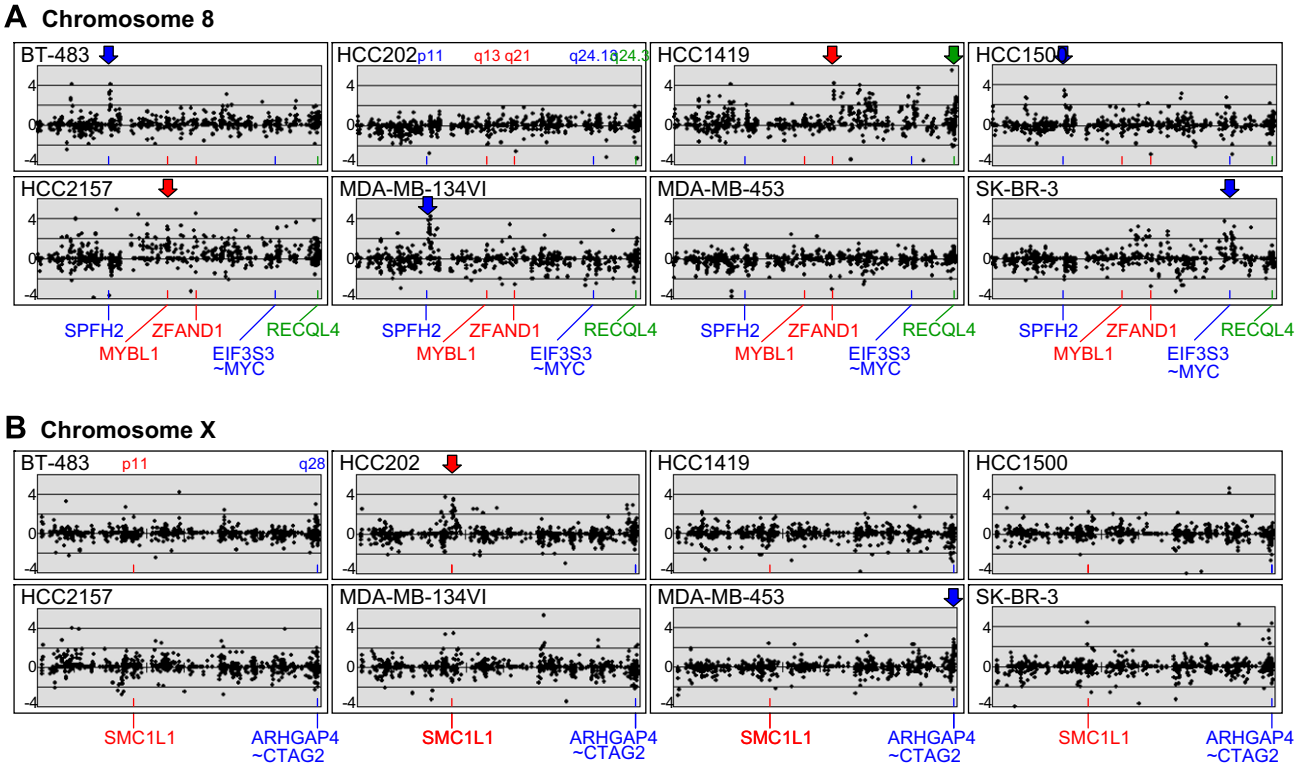

Fig. 3. Mapping of gene expression data on chromosomes 8 (A) and X (B). The maximum values on the horizontal axis are proportionally equivalent to the sizes of the chromosomes: 147 Mb (A); 155 Mb (B). For arrow indications, refer to the legend for Fig. 1. The panel for HCC202 or BT-483 cells includes the number of bands for each chromosome (see Supplementary Figs. 2 and 3 in terms of 27 other "amplicon-negative" cell lines).

Xp11 in HCC202 cells (Fig. 3, red arrows). Moreover, an amplicon candidate detected at 8p24.3 in HCC1419 cells, containing *RECQL4*, has not been identified in breast cancer so far but reported in metastasized colorectal cancers previously (Fig. 3, green arrow) [19]. Additionally, we detected the following previously identified amplicons: at 8p11, containing *SPFH2* (*C8orf2*), in BT-483, HCC1500, and MDA-MB-134VI cells; at 8q24.13, specifically between *EIF3S3* and *MYC*, in SK-BR-3 cells; and at Xq28, specifically between *ARHGAP4* and *CTAG2*, in MDA-MB-453 cells (Fig. 3, blue arrows) [1,2,20,21]. Fig. 4 shows detailed descriptions of subtracted log ratio values for the novel amplicon candidates in chromosomes 8 and X.

Subsequently, we investigated the novel amplicon candidates detected in this study in chromosomes 8, 17, and X by using Southern blot analyses with genomic DNA extracted from the corresponding cell lines (Fig. 5). First, Southern blot analyses with the probe *SPFH2* within 8p11 and a previously identified amplicon revealed amplification in the genomic DNA extracted from BT-483, HCC1500, and MDA-MB-134VII; this was entirely consistent with the results obtained on exploring the gene expression profiles and previous reports[1,2]. Next, Southern blot analyses of the novel amplicon candidates identified in this study were performed to examine amplification in each genomic region for the corresponding cell lines as follows: the probe *HCP1* for 17q11 in ZR-75-30 cells; the probe *EPN3* for 17q21 in BT-474, HCC202, and HCC2218 cells; the probe *C17orf28* for 17q25 in MDA-MB-453 cells; the probe *SMC1L1* for Xp11 in HCC202 cells; the probe *LLGL2* for 17q25 in HCC2218 and UACC893 cells; and the probe *ZFAND1* for 8q21 in HCC1419 cells. We used two independent probes for 17q25 since this region seemed to be divided into two subregions: one containing *C17orf28* and the other containing *LLGL2* (the interval between the initiation sites of *C17orf28* and *LLGL2* was approximately 0.6 Mbp) (Fig. 2D). Comparison of band intensities among cell lines supported gene amplification of these loci and proved reliability of our screening algorithm (compare Fig. 5 with Figs. 1, 3 and supplementary Figs. 1–3).

Further, we tested whether such genomic amplification was present in breast cancer tissues. Initial Southern blot analyses of DNA from 33 breast cancer tissues revealed that the regions 8q21 and 17q21 were significantly amplified in one tumor specimen from each breast cancer tissue, eliminating the possibility that gene amplification occurred during the establishment of the cell lines (Fig. 6). In the present study, since the frequency of amplification and sample numbers were insufficient, the amplified regions were not statistically evaluated. Extensive works of more clinical samples are required to elucidate the significance of the amplification; nevertheless, maintenance of the amplified regions may confer an evolutionary advantage to cancers.

Of the five highly expressed genes at 8q21, *ZFAND1* codes for an uncharacterized protein with an AN1-type zinc finger domain that is found in the DNA-binding, ATP-dependent helicase protein SMUBP-2 (Fig. 4B). *IMPA1* codes for inositol(myo)-1(or 4)-monophosphatase 1, which is involved in inositol phosphate metabolism. Epsin 3 encoded by *EPN3* at 17q21 is localized specifically to migrating keratinocytes in cutaneous wounds but is not found in intact skin, suggesting

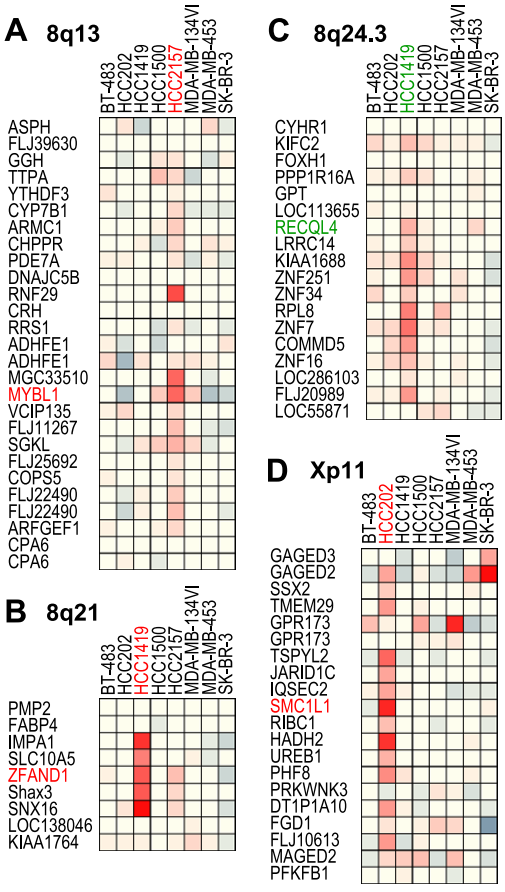

Fig. 4. Relative expression levels of genes localized in amplicon candidates detected in chromosomes 8 and X. Expression values (subtracted log ratios) of the genes localized at 8q13 (A), 8q21 (B), 8q24.3 (C), and Xp11 (D) are represented in color. Cell lines depicted in red and green contain spike-like clusters shown in Fig. 3 as red or green arrows. Genes are aligned in order of chromosome location. For color indications, refer to the legend for Fig. 2.

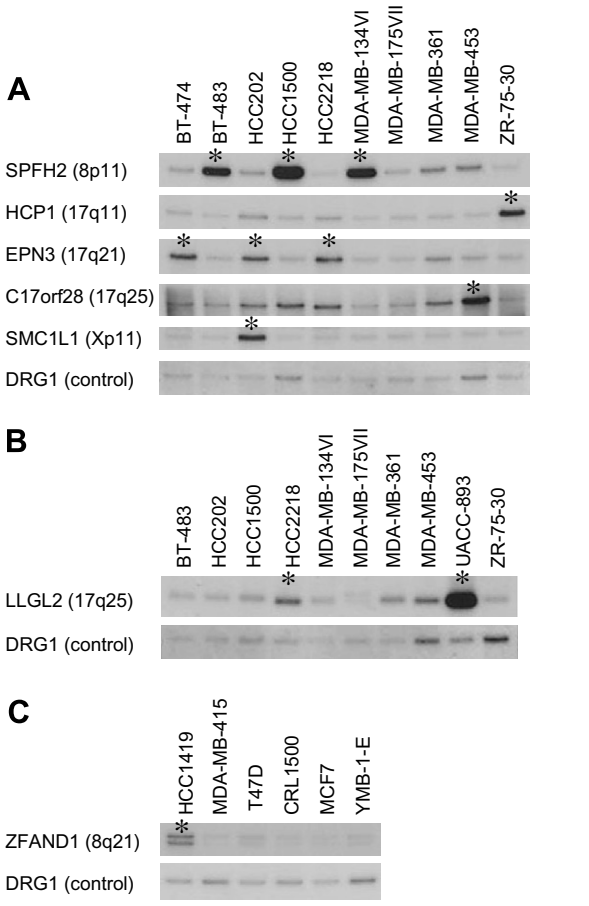

Fig. 5. Southern blot analyses of genomic amplification in breast cancer cell lines. Five micrograms of genomic DNA was subjected to Southern blot analyses. cDNA fragments for probes were amplified by PCR and then cloned into pGEM-T easy (Promega) for labeling. Information regarding each probe is available from the corresponding author. Human *DRG-1* cDNA was used as a control. The asterisk depicts the cell line with confirmed amplification.

that *EPN3* may function in activated epithelial cells during tissue morphogenesis (Fig. 2C) [22]. *ABCC3* codes for an ATP-binding cassette subfamily C that is also known as multidrug resistance-associated protein 3 (MRP3). MRP3 confers resistance to etoposide and teniposide [23].

In the post-sequencing era, innovative tools are needed to extract biologically significant information from extensive gene localization and expression data. Microarray-based CGH has been widely used to detect imbalanced chromosomal regions and has contributed to the identification of novel oncogenes. Although similar studies have been conducted extensively over a long period [24,25], we successfully detected six novel amplicon candidates from only three chromosomes (8, 17, and X) among 35 cell lines in addition to two amplicons previously reported in other cancers but not yet identified in breast cancer (summarized in Table 1). Our approach only requires a simple comparison of an expression ratio obtained from each sample with the mean average of the expression ratios across all samples for each gene. We think our method will be applicable when enough number of samples that share common background are collected. Our system is expected to be useful also for clinical specimen. Discovery of novel gene clusters with aberrant expression levels and subsequent identification of

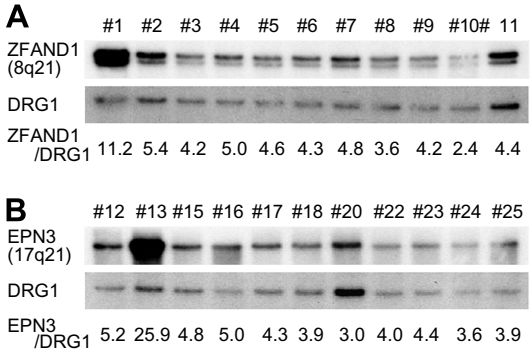

Fig. 6. Southern blot analyses of genomic amplification in breast cancer specimens. Five micrograms of genomic DNA from tumor tissues was subjected to Southern blot analysis. *DRG1* probe was used as a control. Each number (#1 to #25) indicates the patient code number. Relative signal ratios, *ZFAND1*/*DRG1* and *EPN3*/*DRG1*, were calculated by normalizing the signal intensities of *ZFAND1* and *EPN3* to that of *DRG1* measured with a BAS2000 (FUJIFILM, Japan). Human breast cancer specimens from tumors were obtained at Dokkyo Medical University Hospital (Japan). The Ethics Committee of Dokkyo Medical University approved this study, and informed consent was obtained from all patients.

Table 1
Summary of this study

Ch	Probe	Cell line		Tissue
		Map	Southern	Southern
8q13	MYBL1	HCC2157	ND	ND
8q21	ZFAND1	HCC1419	Confirmed	1/33
17q11	HCP1	ZR-75-30	Confirmed	0/33
17q21	EPN3	BT-474	Confirmed	1/33
		HCC202	Confirmed	
		HCC2218	Confirmed	
17q25	C17orf28	MDA-MB-453	Confirmed	ND
	LLGL2	HCC2218	Confirmed	0/33
		UACC-893	Confirmed	
Xp11	SMC1L1	HCC202	Confirmed	0/33

Ch, chromosome; ND, not done.

the genes responsible for cancer malignancy will provide targets for efficacious anticancer therapies.

Acknowledgements: We thank M. Hashimoto for the secretarial assistance. This work was supported in part by the "Establishment of Consolidated Research Institute for Advanced Science and Medical Care"; Encouraging Development Strategic Research Centers Program, Ministry of Education, Culture, Sports, Science and Technology, Japan; and New Energy and Industrial Technology Development Organization (NEDO).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.07.016.

References

[1] Garcia, M.J. et al. (2005) A 1 Mb minimal amplicon at 8p11-12 in breast cancer identifies new candidate oncogenes. Oncogene 24, 5235–5245.
[2] Gelsi-Boyer, V. et al. (2005) Comprehensive profiling of 8p11-12 amplification in breast cancer. Mol. Cancer Res. 3, 655–667.
[3] Hughes-Davies, L. et al. (2003) EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell 115, 523–535.
[4] Slamon, D.J. et al. (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244, 707–712.
[5] Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A. and McGuire, W.L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235, 177–182.
[6] Ross, J.S. et al. (2003) The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. Oncologist 8, 307–325.
[7] Ross, J.S. and Fletcher, J.A. (1998) The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Oncologist 3, 237–252.
[8] Pegram, M.D. et al. (1998) Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J. Clin. Oncol. 16, 2659–2671.
[9] Baselga, J. et al. (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J. Clin. Oncol. 14, 737–744.
[10] Mu, D. et al. (2003) Genomic amplification and oncogenic properties of the KCNK9 potassium channel gene. Cancer Cell 3, 297–302.
[11] Luoh, S.W., Venkatesan, N. and Tripathi, R. (2004) Overexpression of the amplified Pip4k2b gene from 17q11-12 in breast cancer cells confers proliferation advantage. Oncogene 23, 1354–1363.
[12] Cheng, K.W. et al. (2004) The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers. Nat. Med. 10, 1251–1256.
[13] Miura, A. et al. (2006) Differential responses of normal human coronary artery endothelial cells against multiple cytokines comparatively assessed by gene expression profiles. FEBS Lett. 580, 6871–6879.
[14] Wain, H.M., Lush, M.J., Ducluzeau, F., Khodiyar, V.K. and Povey, S. (2004) Genew: the human gene nomenclature database, 2004 updates. Nucleic Acids Res. 32, D255–D257.
[15] Rosen, N., Chalifa-Caspi, V., Shmueli, O., Adato, A., Lapidot, M., Stampnitzky, J., Safran, M. and Lancet, D. (2003) GeneLoc: exon-based integration of human genome maps. Bioinformatics 19 (Suppl. 1), i222–i224.
[16] Kauraniemi, P., Barlund, M., Monni, O. and Kallioniemi, A. (2001) New amplified and highly expressed genes discovered in the ERBB2 amplicon in breast cancer by cDNA microarrays. Cancer Res. 61, 8235–8240.
[17] Hyman, E. et al. (2002) Impact of DNA amplification on gene expression patterns in breast cancer. Cancer Res. 62, 6240–6245.
[18] Meza-Zepeda, L.A. et al. (2006) Array comparative genomic hybridization reveals distinct DNA copy number differences between gastrointestinal stromal tumors and leiomyosarcomas. Cancer Res. 66, 8984–8993.
[19] Buffart, T.E. et al. (2005) DNA copy number changes at 8q11-24 in metastasized colorectal cancer. Cell Oncol. 27, 57–65.
[20] Savinainen, K.J., Linja, M.J., Saramaki, O.R., Tammela, T.L., Chang, G.T., Brinkmann, A.O. and Visakorpi, T. (2004) Expression and copy number analysis of TRPS1, EIF3S3 and MYC genes in breast and prostate cancer. Br. J. Cancer 90, 1041–1046.
[21] Glinsky, G.V., Ivanova, Y.A. and Glinskii, A.B. (2003) Common malignancy-associated regions of transcriptional activation (MARTA) in human prostate, breast, ovarian, and colon cancers are targets for DNA amplification. Cancer Lett. 201, 67–77.
[22] Spradling, K.D., McDaniel, A.E., Lohi, J. and Pilcher, B.K. (2001) Epsin 3 is a novel extracellular matrix-induced transcript specific to wounded epithelia. J. Biol. Chem. 276, 29257–29267.
[23] Zelcer, N., Saeki, T., Reid, G., Beijnen, J.H. and Borst, P. (2001) Characterization of drug transport by the human multidrug resistance protein 3 (ABCC3). J. Biol. Chem. 276, 46400–46407.
[24] Kano, M., Nishimura, K., Ishikawa, S., Tsutsumi, S., Hirota, K., Hirose, M. and Aburatani, H. (2003) Expression imbalance map: a new visualization method for detection of mRNA expression imbalance regions. Physiol. Genom. 13, 31–46.
[25] Carter, S.L., Eklund, A.C., Kohane, I.S., Harris, L.N. and Szallasi, Z. (2006) A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat. Genet. 38, 1043–1048.